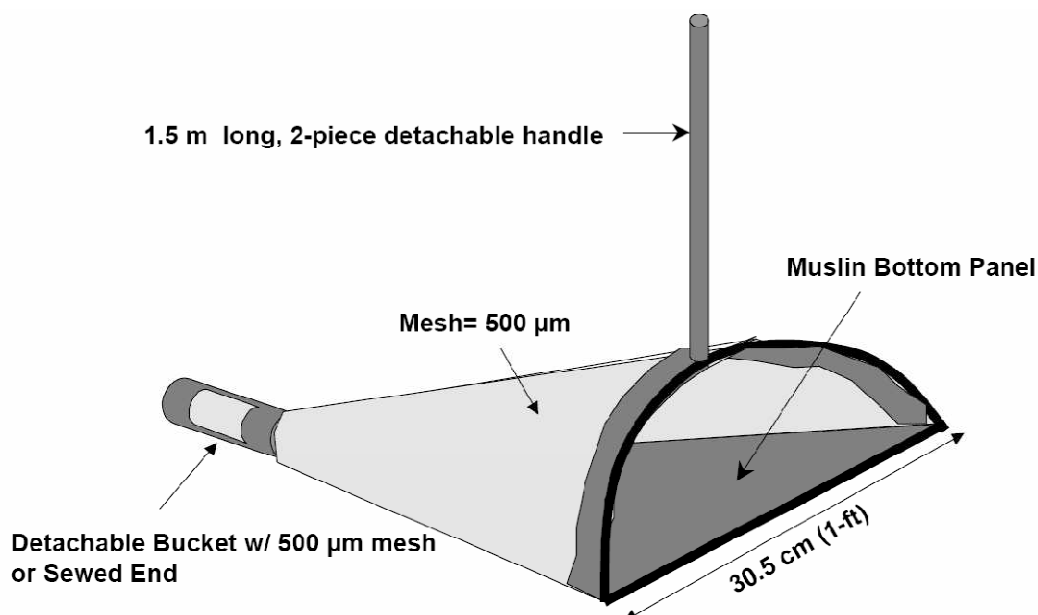7.17

STANDARD OPERATING PROCEDURES FOR THE COLLECTION OF A MACROINVERTEBRATE SAMPLE FROM WADEABLE RIVERS AND STREAMS

Summary

Benthic macroinvertebrates inhabit the sediment or live on the bottom substrates of streams. This group of organisms is an important component for measuring the overall biological condition of an aquatic community. Populations in the benthic assemblage respond to a wide array of stressors in different ways. By monitoring assemblage status it is possible to detect trends in ecological condition and often determine the type of stress that has affected a macroinvertebrate community (e.g., Klemm et al., 1990). Because many macroinvertebrates have life cycles of a year or more and are relatively immobile, the structure and function of the macroinvertebrate assemblage is a response to exposure of past or present conditions.

This benthic macroinvertebrate protocol is intended to evaluate the biological condition of wadeable streams in North Dakota for the purpose of detecting stresses on community structure and assessing the relative severity of these stresses. It is based on the updated Rapid Bioassessment Protocols (RBPs) and Wadeable Streams Assessment (WSA), both published by the U.S. Environmental Protection Agency (Barbour et al., 1999), and adopted for use by many states. The protocol's use of a D-frame kick net (Figure 7.17.1) for benthic macroinvertebrate collection is versatile for varying habitat type and is the preferred macroinvertebrate collecting method for streams with flowing water.

1.5 m long, 2-piece detachable handle →

Muslin Bottom Panel

Mesh= 500 μm

Detachable Bucket w/ 500 μm mesh or Sewed End

30.5 cm (1-ft)

Figure 7.17.1. Modified D-frame Kick Net (Not drawn to scale)

Sampling Period

The index period for macroinvertebrate collection is July-September of each year. This index period will allow macroinvertebrate collections to be taken during normal, stable flow conditions each season.

Equipment and Supply Checklist

All supplies listed should be considered required unless otherwise noted. During the project planning phase, inventory and organize field equipment and supplies. To conduct activities efficiently, examine the list prior to leaving for a stream site.

Equipment List

√	ITEM	QTY
	Surveyor's rod, tape measure, or laser range finder	1
	Modified kick net (D-frame with 500 μm mesh) and 4-ft handle (Wildco #425-C50)	1
	Buckets, plastic, 8- to 10-qt capacity	2
	Hand sieve with 500 μm mesh openings	1
	Watchmakers' forceps	2
	Wash bottle, 1-L capacity labeled "STREAM WATER"	1
	Small spatula, spoon, or scoop to transfer sample	1
	Funnel, with large bore spout	1
	Sample jars, HDPE plastic with screw caps, 500-mL and 1-L capacity, suitable for use with ethanol	1 to 3
	95% Ethanol, in a proper container	2 gal
	Rubber gloves, heavy rubber	2 pr.
	Cooler (with suitable absorbent material) for transporting ethanol and samples	1
	Composite Benthic sample labels, with preprinted ID numbers (barcodes)	2
	Composite Benthic sample labels without preprinted ID numbers	4
	Blank labels on waterproof paper for inside of jars	3
	Sample collection form for site	1
	Clear mailing tape (strips or roll)	1 pkg.
	Pocket knife	1
	Scissors	1
	Pocket-sized field notebook (optional)	1
	Field operations and methods manual	1 copy
	Laminated sheets of procedure tables and/or quick reference guides for benthic macroinvertebrates	1 set
	Spare net(s) and/or spare bucket assembly for end of net	Optional
	Sieve-bottomed bucket, 500 μm mesh openings	Optional
	Soft (#2) lead pencils	
	Fine-tip indelible markers	

Procedures

Lay Out of the Sampling Reach

1. Use a surveyor's rod, tape measure, or laser range finder to determine the wetted width of the channel at 5 places of "typical" width within approximately 5 channel widths upstream and downstream from the X-site. Average the 5 readings together and round to the nearest 1 m. If the average width is <4 m, use 150 m as a minimum reach length. If the average width is >12.5 m, use 500 m as a maximum reach length. *For channels with "interrupted flow", estimate the width based on the unvegetated width of the channel (again, with a 150 m minimum and 500 m maximum).*

2. Check the condition of the stream about the X-site by having one team member go upstream and one downstream. Each person proceeds until they can see the stream to a distance of 20 times the average channel width (equal to one-half the sampling reach length) determined in Step 1.

3. Determine if the reach needs to be adjusted about the X-site due to confluences with higher order streams (downstream), a change to a lower order streams (upstream), impoundments (lakes, reservoirs, ponds), physical barriers (e.g., falls, cliffs), or because of access restrictions to a portion of the initially-determined sampling reach.

4. Starting at the X-site (or the new midpoint of the reach if it had to be adjusted as described in Step 3), measure a distance of 20 channel widths down one side of the stream using a GPS unit, laser rangefinder, or tape measure. Be careful not to "cut corners". Enter the channel to make measurements only when necessary to avoid disturbing the stream channel prior to sampling activities. This endpoint is the downstream end of the reach, and is flagged as Transect "A".

5. At Transect A, use the seconds display on a digital watch to select the initial sampling station for standard transect samples: 1-3="Left", 4-6="Center", 7-9=Right. Mark "L", "C", or "R" on the transect flagging; the 3 potential collection points are roughly equivalent to 25%, 50%, and 75% of the channel width, respectively.

6. Measure 1/10 of the required reach length upstream from transect A. Flag this spot as transect B. Assign the sampling station systematically after the first random selection (Figure 4-6 & Table 4-6).

7. Proceed upstream with the tape measure and flag the positions of 9 additional transects (labeled "C" through "K" as you move upstream) at intervals equal to 1/10 of the reach length. Continue to assign the sampling stations systematically.

Sample Collection

The transect sample design for collecting benthic macroinvertebrates is shown in Figure 7.17.2. This design was used in the Environmental Monitoring & Assessment Program (EMAP) - Western Pilot stream study in the western U.S., which provides continuity for nationwide assessments.

A sample is collected from **1 meter downstream** of each of eleven cross-section transects (Transects "A" through "K") at an assigned area (Left, Center, or Right in the stream). The sampling point at Transect "A" is assigned at random using a die or other suitable means (e.g., digital watch). Once the first sampling point is determined, points at successive transects are assigned in order (Left, Center, Right). At transects assigned a "Center" sampling point where the stream width is between one and two net widths wide, pick either the "Left" or "Right" sampling point instead. If the stream is only one net wide at any transect, place the net across the entire stream width and consider the sampling point to be "Center". If a sampling point is located in water that is too deep or otherwise unsafe to wade, select an alternate sampling point on that transect.

The procedure for sample collection at each transect is described below. For each sampling site, first establish that the majority of the river reach is wadeable. Next determine if the habitat is a "riffle/run" or a "pool/glide". Any area where water current is not sufficient to extend the net is operationally defined as a pool/glide habitat. As sampling proceeds upstream from transect to transect, combine all samples into a bucket or similar container.

Do not fill out the collection form or labels until wadeablity is confirmed at the site and samples are collected.

1. At **1 m downstream** of each cross-section transect, beginning with Transect "A", locate the assigned sampling point (Left, Center, or Right facing downstream) at 25%, 50%, and 75% of the wetted width, respectively. If a sample cannot be collected at the designated point because of deep water or unsafe conditions, relocate to another random point on the same transect.

2. Make sure that the D-frame net is securely tightened to the pole as the net may become twisted in a strong current, causing the loss of sample materials.

3. Determine if there is sufficient current in the area at the sampling point to fully extend the net. If so, classify the habitat as "riffle/run" and proceed to Step 4. If not, use the sampling procedure described for "pool/glide" habitats, Step 9.

    NOTE: If large rocks prevent the sampler from seating the net properly on the stream bottom, spend 30 seconds hand picking a sample from about 0.09 m^2 (1 ft^2) of substrate at the sampling point. For vegetation-choked sampling points, sweep the net through the

vegetation within a 0.09 m^2 (1 ft^2) quadrat for 30 seconds. Place the contents of this hand-picked sample directly into the sampling container.

Riffle/Run Habitats:

4. With the net opening facing upstream, position the net quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid large rocks that prevent the sampler from seating the net properly on the stream bottom.

    NOTE: If there is too little water to collect the sample with the D-frame net, randomly pick up 10 rocks from the riffle. Wash anything attached to the rocks (e.g. sand, insects, vegetation, etc.) into the closed or sieve bottomed bucket and pick any remaining organisms off and place them into the bucket.
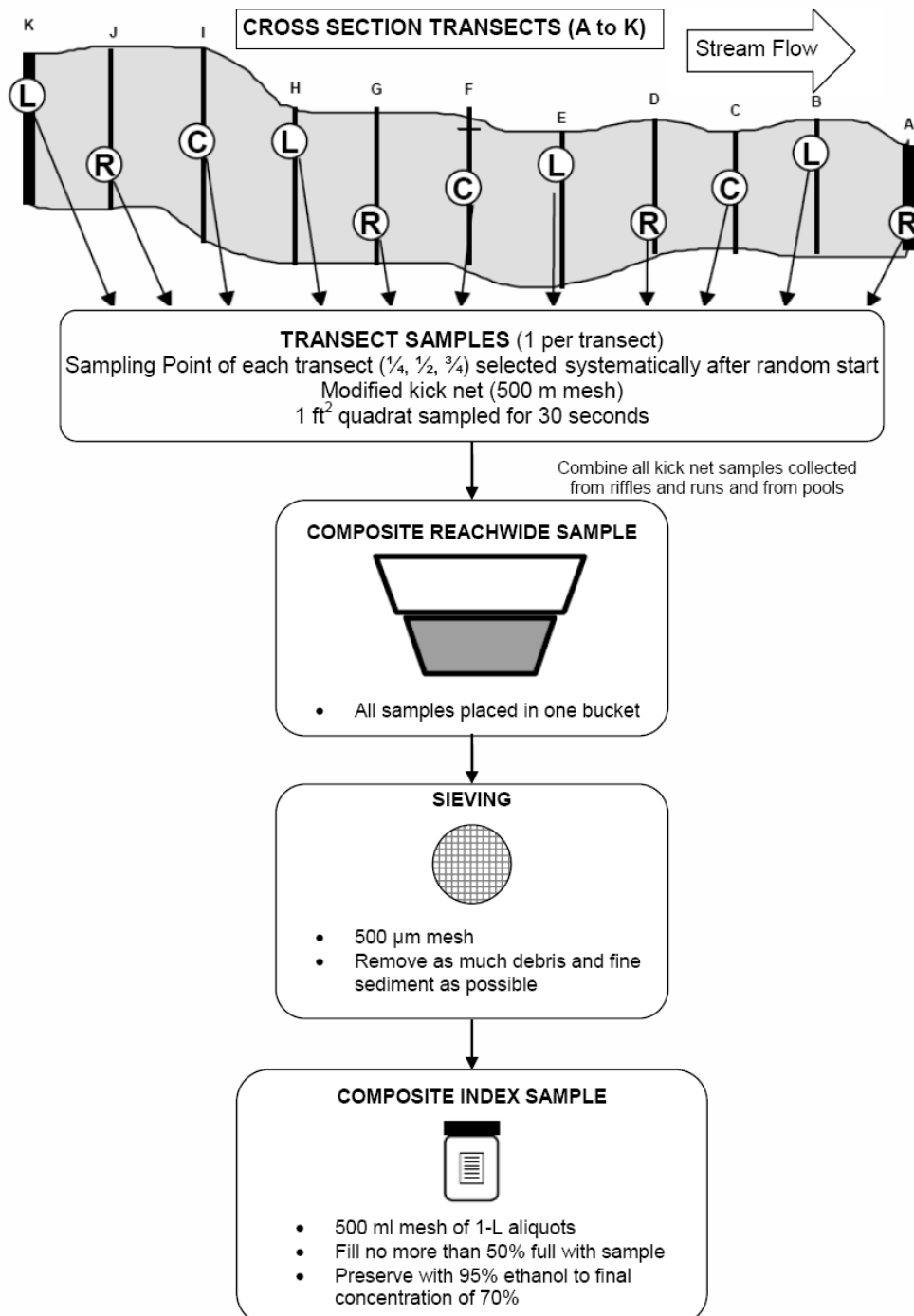
5. While holding the net in position on the substrate, visually define a rectangular quadrat that is one net width wide and one net width long upstream of the net opening. The area within this quadrat is 0.09 m^2 (1 ft^2).

6. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms from the substrate by hand and place them into the net. Pick up any loose rocks or other larger substrate particles in the quadrat. Use hands or a small scrub brush to dislodge organisms so that they are washed into the net. Scrub all rocks that are golf ball-sized or larger and which are over halfway into the quadrat. Large rocks that are less than halfway into the sampling area should be pushed aside. After scrubbing, place the substrate particles outside of the quadrat.

7. Keep holding the D-net securely in position. Start at the upstream end of the quadrat; vigorously kick the remaining finer substrate within the quadrat for 30 seconds (use a stopwatch).

    NOTE: For samples located within dense beds of long, filamentous aquatic vegetation (e.g., algae or moss), kicking within the quadrat may not be sufficient to dislodge organisms in the vegetation. Usually these types of vegetation are lying flat against the substrate due to current. Use a knife or scissors to remove **only the vegetation that lies within the quadrat** (i.e., not entire strands that are rooted within the quadrat) and place it into the net.

8. Pull the net up out of the water. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water or material enter the mouth of the net during this operation. **Go to Step 13**.

Pool/Glide habitats:

9. Visually define a rectangular quadrat that is one net width wide and one net width long at the sampling point. The area within this quadrat is -0.09 m^2 (1 ft^2).

CROSS SECTION TRANSECTS (A to K)

Stream Flow

TRANSECT SAMPLES (1 per transect)
Sampling Point of each transect (¼, ½, ¾) selected systematically after random start
Modified kick net (500 m mesh)
1 ft^2 quadrat sampled for 30 seconds

Combine all kick net samples collected
from riffles and runs and from pools

COMPOSITE REACHWIDE SAMPLE

- All samples placed in one bucket

SIEVING

- 500 µm mesh
- Remove as much debris and fine
  sediment as possible

COMPOSITE INDEX SAMPLE

- 500 ml mesh of 1-L aliquots
- Fill no more than 50% full with sample
- Preserve with 95% ethanol to final
  concentration of 70%

Figure 7.17.2. Transect Sampling Design for the Benthic Macroinvertebrate Sample

10. Inspect the stream bottom within the quadrat for any heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net or bucket. Pick up any loose rocks or other larger substrate particles within the quadrat and hold them in front of the net. Use hands (or a scrub brush) to rub any clinging organisms off of rocks or other pieces of larger substrate (especially those covered with algae or other debris) into the net. After scrubbing, place the larger substrate particles outside of the quadrat.

    NOTE: If the water is too deep to effectively "kick" the substrate in front of the net, face upstream and jab/sweep the net through the $0.09m^2$ quadrat. So that individuals from previous jabs do not escape the net after each sequence, remove the net completely from the water and replace it at the start of the quadrat. Three series of jabs/sweeps through the quadrat should be sufficient to obtain a sample at each transect.

11. Vigorously kick the remaining finer substrate within the quadrat while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net all the time so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds.

    NOTE: If there is too little water to use the kick net, stir up the substrate with gloved hands and use a sieve with 500 μm mesh size to collect the organisms from the water in the same way the net is used in larger pools.

12. After 30 seconds, remove the net from the water with a quick, net surfacing motion to wash the organisms to the bottom of the net.

All samples:

13. Invert the net into a bucket and transfer the sample. Inspect the net for any organisms clinging to the mesh and use forceps to deposit them into the bucket. Carefully inspect any large objects (e.g. rocks, sticks, or leaves) in the bucket and wash any organisms into the bucket. Discard as much rocks, sediments and detritus as possible without losing any organisms.
14. Thoroughly rinse the net before proceeding to the next sampling location. Proceed upstream to the next transect (including Transect K, the upstream end of the sampling reach) and repeat Steps 1 through 14. Combine all kick net samples from riffle/run and pool/glide habitats into the bucket.

Sample Processing

Use a closed bottom or sieve bottom bucket while sampling to carry the composite sample to the next transect. Alternatively, place each sample in a five-gallon bucket and use a soil sieve (500 μm = 0.5 mm) to cull-down the sample before it is packed and preserved in a Nalgene container(s) upon completion (Figure 7.17.2). Record the composite sample tracking information on a field recording form shown in Figure 7.17.3.

1. Pour the entire contents of the bucket through a sieve (or into a sieve bucket) with 500 μm mesh size. Remove any large objects and wash any clinging organisms back into the sieve before discarding.

2. Use a wash bottle filled with stream water to rinse all the organisms from the bucket into the sieve. This is the composite reach-wide sample for the site.

3. Estimate the total volume of the sample in the sieve and determine how large a jar will be needed for the sample (500-mL or 1-L) and how many jars will be required.

4. Wash the contents of the sieve to one side by gently agitating the sieve in the water. Wash the sample into a jar using as little water from the wash bottle as possible. Use a large-bore funnel if necessary. If the jar is too full pour off some water through the sieve until the jar **is not more than ½ full**, or use a second jar if a larger one is not available. Carefully examine the sieve for any remaining organisms and use watchmakers' forceps to place them into the sample jar.

5. Completely fill the jar(s) with 95% ethanol (no headspace). It is very important that sufficient ethanol be used or the organisms will not be properly preserved.

   NOTE: Prepared composite samples can be transported back to the vehicle before adding ethanol if necessary. Fill the jar with stream water, which is then drained using the net across the opening to prevent loss of organisms, and replaced with ethanol.

6. Place a waterproof label (Figure 7.17.4), with the following information, inside of each jar. **Use a lead pencil when writing waterproof label as ink will deteriorate in Ethanol.**

   • Project Code   • Site Identification number
   • Type of sample   • Number of transect samples composited (Jar N of X)
   • Sample container size   • Preservation fixative
   • Date of collection   • Time of collection
   • Sampler Name

   NOTE: Write "Jar *N* of *X*" on each sample label using a waterproof marker ("*N*" is the individual jar number, and "*X*" is the total number of jars for the sample).

7. Replace the cap on each jar. Slowly tip the jar to a horizontal position, and then gently rotate the jar to mix the preservative. Do not invert or shake the jar.

8. Fill in outside jar labels with the stream information listed in step 6. Attach the completed label to the jar and cover it with a strip of clear mailing tape. Record the sample information for the composite sample on the Sample Log Form (Figure 7.17.5). For each composite sample, make sure the number on the form matches the number on the label.

9. Store labeled composite samples in a container with absorbent material that is suitable for use with 95% ethanol until transport or shipment to the laboratory.

LITERATURE CITED

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Klemm, D.J., J.M. Lazorchak, and P.A. Lewis. 1998. Benthic Macroinvertebrates. pp. 147- 182 IN: J.M. Lazorchak, D.J. Klemm, and D.V. Peck (Eds.). *Environmental Monitoring and Assessment Program-Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams*. EPA/620/R-94/004F. U.S. Environmental Protection Agency, Washington, D.C.

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Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish.* EPA/440/4-89/001. U.S. Environmental Protection Agency, Assessment and Watershed Protection Division, Washington, D.C.

Field Recording Form for Biological Monitoring
North Dakota Department of Health
Division of Water Quality-SWQMP
Telephone: 701.328.5210
Fax: 701.328.5200

SITE ID:_____ **DATE:** _____/ _____/ _____

FIELD NUMBER:_____ **SAMPLERS:** _____

STATION DESCRIPTION:_____

_____

LATITUDE: _____ **LONGITUDE:** _____

ECOREGION (circle one)**:**   43   42   46   48

INVERTEBRATE COLLECTION METHOD (circle one):   D-NET   OTHER _____

REACH LENGTH:_____ **M**

STREAM HABITAT TYPE (%):	RIFFLE:_____	POOL:_____	SNAG:_____	UNDERCUT BANK:_____
	AQUATIC VEG: _____	OVERHANG VEG:_____	OTHER:_____	

FIELD WATER CHEMISTRY	SITE PHOTOS
TEMP:	UPSTREAM:
DO:	DOWNSTREAM:
pH:	
COND:	

WEATHER CONDITIONS (Temp., Wind, etc.): _____
_____
COMMENTS: _____
_____

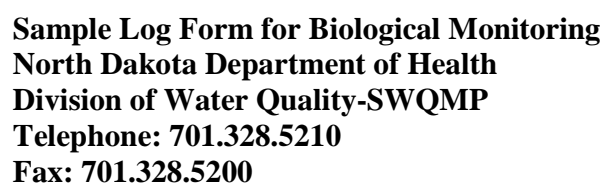**Figure 7.17.3.** Macroinvertebrate Field Collection Data Recording Form

SITE DRAWING (Show direction of water flow and north)

COMMENTS:

Figure 7.17.3 ctd. Macroinvertebrate Field Collection Data Recording Form (reverse).

Project Code_____   Site ID#:_____

Site#:_____   Field#:_____

Analysis: Macroinvertebrates   Jar_____ of _____
Container: 1L   Preservation: 95% Ethanol

Date_____/_____/_____   Time_____:_____

Sampler:

Project Code_____   STORET#:_____

Site#:_____   Field#:_____

Analysis: Macroinvertebrates   Jar_____ of _____
Container: 1L   Preservation: 95% Ethanol

Date_____/_____/_____   Time_____:_____

Sampler:

Project Code_____   STORET#:_____

Site#:_____   Field#:_____

Analysis: Macroinvertebrates   Jar_____ of _____
Container: 1L   Preservation: 95% Ethanol

Date_____/_____/_____   Time_____:_____

Sampler:

Project Code_____   STORET#:_____

Site#:_____   Field#:_____

Analysis: Macroinvertebrates   Jar_____ of _____
Container: 1L   Preservation: 95% Ethanol

Date_____/_____/_____   Time_____:_____

Sampler:

Figure 7.17.4. Macroinvertebrate Sample Jar Labels (both inside and outside).

Sample Log Form for Biological Monitoring
North Dakota Department of Health
Division of Water Quality-SWQMP
Telephone: 701.328.5210
Fax: 701.328.5200

Field Number	Station ID and Description	Date/Time	Collection Method	Comments

Figure 7.17.5. Macroinvertebrate Sample Log.